

CLAIMS

1. An isolated cat protein allergen, human T cell reactive feline protein,
5 (TRFP) comprising two covalently linked peptide chains.

2. The isolated cat protein allergen of claim 1, wherein said two covalently linked peptide chains comprise the amino acid sequences of chain 1 and chain 2 shown in Figs. 6 and 7 (SEQ ID NOS:2, 4, 6 and 8).

10 3. An isolated cat protein allergen of claim 1, wherein each of said peptide chains is produced by recombinant expression of a nucleic acid comprising a nucleotide sequence encoding each of said peptide chains.

15 4. The isolated cat protein allergen of claim 1, which is essentially free of all other cat proteins.

5. The isolated cat protein allergen of claim 3, wherein the nucleic acid is a cDNA sequence selected from the group consisting of:

20 (a) the cDNA sequence encoding chain 1, leader A (SEQ ID NO:1), as represented in Fig. 7;

(b) the cDNA sequence encoding chain 1, leader B, (SEQ ID NO:3) as represented in Fig. 2;

25 (c) the cDNA sequence encoding chain 2, long form, (SEQ ID NO:5) as represented in Fig. 3;

(d) the cDNA sequence encoding chain 2, short form, (SEQ ID NO:7) as represented in Fig. 4; and

(e) the cDNA sequence encoding chain 2, truncated sheet form, (SEQ ID NO:9) as represented in Fig. 5.

30 6. The isolated cat protein of claim 5 which is produced in *E. coli* as a recombinant fusion protein.

7. An isolated peptide of human T cell reactive feline protein (TRFP) comprising a sequence of amino acid residues selected from the group consisting of:

- (a) TRFP chain 1, leader A, as represented in Fig. 6 (SEQ ID NO:2);
- (b) TRFP chain 1, leader B, as represented in Fig. 6 (SEQ ID NO:4);

(c) TRFP chain 2, long form, as represented in Fig. 7 (SEQ ID NO:6);
(d) TRFP chain 2, short form as represented in Fig. 7(SEQ ID NO:8);
(e) TRFP chain 2, short truncated form as represented in Fig. 7(SEQ ID NO:10).

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8. An isolated peptide of human T cell reactive feline protein, said peptide essentially free of all other cat proteins or peptides.

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9. The isolated peptide of claim 8 which stimulates T cells obtained from an individual sensitive to the human T cell reactive feline protein in an *in vitro* T cell proliferation assay.

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10. The isolated peptide of claim 9 having a mean human T cell stimulation index of at least about 4.0 determined in an *in vitro* T cell proliferation assay with T cells obtained from a population of individuals sensitive to the human T cell reactive feline protein.

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11. The isolated peptide of claim 8 having a positivity index of at least about 250 determined in an *in vitro* T cell proliferation assay with T cells obtained from a population of individuals sensitive to the human T cell reactive feline protein.

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12. The isolated peptide of claim 8, comprising at least one T cell epitope of the human T cell reactive feline protein.

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13. The isolated peptide of claim 8, comprising at least two T cell epitopes of the human T cell reactive feline protein.

14. The isolated peptide of claim 8 which is at least about 13-30 amino acids in length.

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15. The isolated peptide of claim 8 which does not bind human immunoglobulin E (IgE) or binds immunoglobulin E to a substantially lesser extent than the naturally occurring TRFP binds IgE.

16. The isolated peptide of claim 13, wherein at least one of the T cell epitopes is an epitope of chain 1 of the human T cell reactive feline protein and at least one of the T cell epitopes is an epitope of chain 2 of the human T cell reactive feline

protein.

17. The isolated peptide of claim 13, wherein the T cell epitopes are epitopes of chain 1 of the human T cell reactive feline protein.

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18. The isolated peptide of claim 13, wherein the T cell epitopes are epitopes of chain 2 of the human T cell reactive feline protein.

19. An isolated peptide of a cat protein allergen, human T cell reactive feline 10 protein, said peptide selected from the group consisting of:

a) amino acids of chain 1 (SEQ. ID. NO: 2) of feline protein, selected from the group consisting of:

15 1) amino acids 1-17, wherein amino acid 3 is T;
2) amino acids 1-17;
3) amino acids 4-17;
4) amino acids 6-17;
5) amino acids 8-17;
6) amino acids 10-17;
7) amino acids 9-25;
8) amino acids 18-33, wherein amino acid 31 is P and amino acid 32 is D;
9) amino acids 18-33;
10) amino acids 18-31;
11) amino acids 18-30;
12) amino acids 18-29;
13) amino acids 18-28;
14) amino acids 18-27;
30 15) amino acids 1-30;
16) amino acids 5-33;
17) amino acids 6-33;
18) amino acids 7-33;
19) amino acids 19-43;
35 20) amino acids 23-36;
21) amino acids 25-36;
22) amino acids 26-36;

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- 23) amino acids 27-42;
- 24) amino acids 29-42;
- 25) amino acids 37-55;
- 26) amino acids 37-52;
- 27) amino acids 37-49;
- 28) amino acids 37-46;
- 29) amino acids 25-49;
- 30) amino acids 25-48;
- 31) amino acids 25-47;
- 32) amino acids 29-55;
- 33) amino acids 29-54;
- 34) amino acids 29-53;
- 35) amino acids 26-55;
- 36) amino acids 28-55;
- 37) amino acids 44-60;
- 38) amino acids 51-66; and
- 39) 56-70, wherein amino acid 70 is R;

20 b) ~~amino acids of chain 2 long (SEQ. ID. NO: 6) of human T cell reactive feline protein, selected from the group consisting of:~~

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1) amino acids 1-22;
2) amino acids 12-33;
3) amino acids 12-24;
4) amino acids 14-24;
5) amino acids 16-24;
6) amino acids 23-48;
7) amino acids 26-36;
8) amino acids 26-38;
30 9) amino acids 26-40;
10) amino acids 14-40;
11) amino acids 14-39;
12) amino acids 14-38;
13) amino acids 14-37;
35 14) amino acids 14-36;
15) amino acids 15-40;
16) amino acids 15-36;

17) amino acids 34-59;
18) amino acids 49-68;
19) amino acids 60-82;
20) amino acids 74-92;

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a) amino acids 60-82 of chain 2 short (SEQ. ID. NO: 8) of human T cell reactive feline protein; and
d) modifications of the amino acid sequences in a), b) or c) above.

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20. An isolated peptide of a cat protein allergen, human T cell reactive feline protein, said peptide comprising a portion of a peptide selected from the group consisting of:

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a) amino acids of chain 1 (SEQ. ID. NO: 2) of feline protein, selected from the group consisting of:

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1) amino acids 1-17, wherein amino acid 3 is T;
2) amino acids 1-17;
3) amino acids 4-17;
4) amino acids 6-17;
5) amino acids 8-17;
6) amino acids 10-17;
7) amino acids 9-25;
8) amino acids 18-33, wherein amino acid 31 is P and amino acid 32 is D;
9) amino acids 18-33;
10) amino acids 18-31;
11) amino acids 18-30;
12) amino acids 18-29;
13) amino acids 18-28;
14) amino acids 18-27;
15) amino acids 1-30;
16) amino acids 5-33;
17) amino acids 6-33;
18) amino acids 7-33;
19) amino acids 19-43;

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20) amino acids 23-36;
21) amino acids 25-36;
22) amino acids 26-36;
23) amino acids 27-42;
24) amino acids 29-42;
25) amino acids 37-55;
26) amino acids 37-52;
27) amino acids 37-49;
28) amino acids 37-46;
29) amino acids 25-49;
30) amino acids 25-48;
31) amino acids 25-47;
32) amino acids 29-55;
33) amino acids 29-54;
34) amino acids 29-53;
35) amino acids 26-55;
36) amino acids 28-55;
37) amino acids 44-60;
38) amino acids 51-66; and
39) 56-70, wherein amino acid 70 is R;

b) amino acids of chain 2 long (SEQ. ID. NO: 6) of human T cell reactive feline protein, selected from the group consisting of:

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- 1) amino acids 1-22;
- 2) amino acids 12-33;
- 3) amino acids 12-24;
- 4) amino acids 14-24;
- 5) amino acids 16-24;
- 6) amino acids 23-48;
- 7) amino acids 26-36;
- 8) amino acids 26-38;
- 9) amino acids 26-40;
- 10) amino acids 14-40;
- 11) amino acids 14-39;
- 12) amino acids 14-38;
- 13) amino acids 14-37;

- 14) amino acids 14-36;
- 15) amino acids 15-40;
- 16) amino acids 15-36;
- 17) amino acids 34-59;
- 18) amino acids 49-68;
- 19) amino acids 60-82;
- 20) amino acids 74-92;

10 c) amino acids 60-82 of chain 2 short (SEQ. ID. NO: 8) of human T cell reactive feline protein; and

d) modifications of the amino acid sequences in a), b) or c) above

15 wherein said peptide stimulates T cells obtained from an individual sensitive to the
human T cell reactive feline protein in an *in vitro* T cell proliferation assay.

21. The isolated peptide of claim 20 having a mean human T cell stimulation index of at least about 4.0 determined in an *in vitro* T cell proliferation assay with T cells obtained from a population of individuals sensitive to the human T cell reactive feline protein.

22. The isolated peptide of claim 20 having a positivity index of at least about 250 determined in an *in vitro* T cell proliferation assay with T cells obtained from a population of individuals sensitive to the human T cell reactive feline protein.

23. The isolated peptide of claim 20, comprising at least two T cell epitopes of the human T cell reactive filaggrin protein.

24. The isolated peptide of claim 20 which is at least about 12 amino acids in length.

25. An isolated peptide comprising at least two regions each having human T cell stimulating activity, said regions each comprising at least one T cell epitope of a cat protein allergen, human T cell reactive feline protein.

35 26. An isolated peptide of claim 25 wherein the regions are arranged in a

human T cell reactive feline protein.

27. An isolated peptide of claim 25 wherein the noncontiguous regions are defined by amino acids and wherein the human T cell reactive feline protein from which the regions are derived comprises amino acids arranged in a sequential order from an amino terminus to a carboxy terminus and wherein said noncontiguous regions of the peptide are arranged in a nonsequential order.

28. An isolated peptide of claim 25 which binds immunoglobulin E to a substantially lesser extent than the native human T cell reactive feline protein from which the regions are derived binds said immunoglobulin E.

29. An isolated peptide of claim 25 wherein the regions are selected from the group consisting of peptide X (SEQ. ID. NO: 17), peptide Y (SEQ. ID. NO: 18), peptide Z (SEQ. ID. NO: 19), peptide A (SEQ. ID. NO: 20), peptide B (SEQ. ID. NO: 21), and peptide C (SEQ. ID. NO: 22).

30. An isolated peptide of claim 29 wherein the regions comprise peptide X (SEQ. ID. NO: 17), peptide Y (SEQ. ID. NO: 18), and peptide Z (SEQ. ID. NO: 19).

31. An isolated peptide AYZXB of claim 29 comprising amino acid residues of peptide A (SEQ. ID. NO: 20), linked in sequential order to amino acid residues of peptide Y (SEQ. ID. NO: 18), amino acid residues of peptide Z (SEQ. ID. NO: 20), amino acid residues of peptide Y (SEQ. ID. NO: 18) and amino acid residues of peptide B (SEQ. ID. NO: 21).

32. An isolated peptide of claim 25 including a proteolytic site inserted between at least two of said regions.

33. A nucleic acid molecule comprising a nucleotide sequence encoding a peptide of claim 25.

34. An isolated peptide comprising an amino acid sequence selected from the group consisting of: Peptide X (SEQ ID NO:17), Peptide Y (SEQ ID NO:18), Peptide Z (SEQ ID NO:19), Peptide A (SEQ ID NO:20, Peptide B (SEQ ID NO:21), Peptide C (SEQ ID NO:22), and Peptide D (SEQ ID NO:23), all as shown in Fig. 18.

35. A composition suitable for pharmaceutical administration comprising a peptide of claim 8.

36. A composition suitable for pharmaceutical administration comprising a peptide of claim 13.

37. A composition suitable for pharmaceutical administration comprising a peptide of claim 19.

10 38. A composition suitable for pharmaceutical administration comprising a peptide of claim 20.

15 39. A composition suitable for pharmaceutical administration comprising a peptide of claim 25.

15 40. A composition suitable for pharmaceutical administration comprising a peptide of claim 34.

20 41. A composition comprising at least two peptides of a cat protein allergen, human T cell reactive feline protein, said peptides selected from the group consisting of peptide X (SEQ. ID. NO: 17), peptide Y (SEQ. ID. NO: 18), peptide Z (SEQ. ID. NO: 19), peptide A (SEQ. ID. NO: 20), peptide B (SEQ. ID. NO: 21), and peptide C (SEQ. ID. NO: 22), all as shown in Fig. 18.

25 42. The composition of claim 41, comprising peptide X (SEQ. ID. NO: 17) and peptide Y (SEQ. ID. NO: 18).

30 43. A composition suitable for pharmaceutical administration comprising a composition of claim 41.

44. A composition suitable for pharmaceutical administration comprising a peptide of claim 42.

35 45. A method of treating sensitivity to a cat protein allergen in a subject sensitive to the allergen, comprising administering to the subject the composition of claim 35.

46. The method of claim 45, wherein the composition is administered subcutaneously.

47. The method of claim 45, wherein the composition is administered orally.

5 48. A method of treating sensitivity to a cat protein allergen in a subject sensitive to the allergen, comprising administering to the subject the composition of claim 37.

10 49. The method of claim 48, wherein the composition is administered subcutaneously.

50. The method of claim 48, wherein the composition is administered orally.

15 51. A method of treating sensitivity to a cat protein allergen in a subject sensitive to the allergen, comprising administering to the subject the composition of claim 34.

20 52. The method of claim 51, wherein the composition is administered subcutaneously.

53. The method of claim 41, wherein the composition is administered subcutaneously.

25 54. A method of treating sensitivity to a cat protein allergen in a subject sensitive to the allergen, comprising Administering to the subject the composition of claim 48.

30 55. The method of claim 54, wherein the composition is administered subcutaneously.

56. The method of claim 54, wherein the composition is administered orally.

35 57. A method of treating sensitivity to a cat protein allergen in a subject sensitive to the allergen, comprising administering to the subject the composition of claim 44.

58. The method of claim 57, wherein the composition is administered subcutaneously.

59. The method of claim 57, wherein the composition is administered orally.

60. A method of treating sensitivity to a cat protein allergen in a subject sensitive to the allergen, comprising administering simultaneously or sequentially to the subject an amount of at least two different compositions of claim 35.

10 61. A method of treating sensitivity to a cat protein allergen in a subject sensitive to the allergen, comprising administering simultaneously or sequentially to the subject an amount of at least two different compositions of claim 37.

15 62. A modified peptide of claim 8 having increased solubility in a pharmaceutically acceptable carrier.

63. A modified peptide of claim 19 having increased solubility in a pharmaceutically acceptable carrier.

20 64. The modified peptide of claim 63, wherein at least one cysteine residue, if present in the peptide, is replaced by another amino acid residue.

25 65. The modified peptide of claim 63, wherein at least one lysine residue is added to either the amino or carboxy terminus or both the amino and carboxy terminus of the peptide.

66. An isolated nucleic acid comprising a nucleotide sequence encoding a cat protein allergen, human T cell reactive feline protein.

30 67. An isolated nucleic acid wherein the nucleic acid is a cDNA sequence, consisting of:

(a) the cDNA sequence encoding chain 1, leader A (SEQ ID NO:1), as represented in Fig. 7;

(b) the cDNA sequence encoding chain 1, leader B, (SEQ ID NO:3) as represented in Fig. 2;

(c) the cDNA sequence encoding chain 2, long form, (SEQ ID NO:5) as represented in Fig. 3;

5 (d) the cDNA sequence encoding chain 2, short form, (SEQ ID NO:7) as represented in Fig. 4; and
(e) the cDNA sequence encoding chain 2, truncated sheet form, (SEQ ID NO:9) as represented in Fig. 5.

68. An isolated nucleic acid comprising a nucleotide sequence encoding a peptide of claim 8.

10 69. An isolated nucleic acid comprising a nucleotide sequence encoding a peptide of claim 10.

70. A recombinant expression vector comprising the nucleic acid of claim 66.

15 71. The recombinant expression vector of claim 70, wherein the nucleic acid is a cDNA sequence selected from the group consisting of:

20 (a) the cDNA sequence encoding chain 1, leader A (SEQ ID NO:1), as represented in Fig. 7;
(b) the cDNA sequence encoding chain 1, leader B, (SEQ ID NO:3) as represented in Fig. 2;
(c) the cDNA sequence encoding chain 2, long form, (SEQ ID NO:5) as represented in Fig. 3;
(d) the cDNA sequence encoding chain 2, short form, (SEQ ID NO:7) as represented in Fig. 4; and
25 (e) the cDNA sequence encoding chain 2, truncated sheet form, (SEQ ID NO:9) as represented in Fig. 5.

30 72. A host cell transfected with the recombinant expression vector of claim 70 capable of directing the expression of a peptide of a cat protein allergen, human T cell reactive feline protein.

73. A method of producing a peptide of a cat protein allergen, human T cell reactive feline protein, comprising culturing a host cell of claim 72 in medium to express the peptide and isolating the peptide from the culture.

35 74. A method of detecting sensitivity in a subject to a cat protein allergen, comprising combining a blood sample obtained from the subject with a protein of claim 1, under conditions appropriate for binding of blood components with the protein and

determining the extent to which such binding occurs.

75. The method of claim 74, wherein the extent to which binding occurs is determined by assessing T cell function, T cell proliferation, B cell function, binding of the protein to antibodies present in the blood or a combination thereof.

76. A method of detecting sensitivity in a subject to a cat protein allergen, comprising combining a blood sample obtained from the subject with a protein of claim 3, under conditions appropriate for binding of blood components with the protein and determining the extent to which such binding occurs.

77. The method of claim 76, wherein the extent to which binding occurs is determined by assessing T cell function, T cell proliferation, B cell function, binding of the protein to antibodies present in the blood or a combination thereof.

78. A method for determining in an individual the presence of immunoglobulin E specific for a cat protein allergen, human T cell reactive feline protein, and the ability of T cells of the individual to respond to T cell epitope(s) of the human T cell reactive feline protein, comprising the steps of:

- 20 a) administering to an individual an Immediate Type Hypersensitivity test utilizing the human T cell reactive feline protein or a portion thereof, or a modified form of the human T cell reactive feline protein or a portion thereof, each of which binds immunoglobulin E specific for the human T cell reactive feline protein;
- 25 b) determining whether a specific Immediate Type Hypersensitivity reaction occurs;
- 30 c) administering to an individual prior to, simultaneously with, or subsequent to administration of the Immediate Type Hypersensitivity test in step (a), a Delayed Type Hypersensitivity test utilizing a modified form of the human T cell reactive feline protein or a portion thereof, or the human T cell reactive feline protein produced by recombinant DNA techniques or a portion thereof, or a peptide comprising at least two regions derived from the human T cell reactive feline protein, or a peptide derived from the human T cell reactive feline protein, each of which has human T cell stimulating activity and each of which does not bind immunoglobulin E specific for the human T cell reactive feline protein, or if binding to said immunoglobulin E occurs, such binding does not result in release of mediators from mast cells or basophils in a substantial percentage of a population of individuals sensitive to the human T cell reactive feline protein; and

d) determining whether a specific Delayed Type Hypersensitivity reaction occurs.

79. A method of detecting and treating sensitivity in an individual to a cat protein allergen, human T cell reactive feline protein, comprising the steps of :

5 a) administering to an individual an Immediate Type hypersensitivity test utilizing the human T cell reactive feline protein or a portion thereof, or a modified form of the human T cell reactive feline protein or a portion thereof, each of which binds immunoglobulin E specific for said at least one protein allergen;

10 b) determining whether a specific Immediate Type Hypersensitivity reaction occurs;

15 c) administering to an individual having a specific Immediate Type Hypersensitivity reaction a Delayed Type Hypersensitivity test utilizing a modified form of the human T cell reactive feline protein or a portion thereof, or the human T cell reactive feline protein produced by recombinant DNA techniques or a portion thereof, or a peptide comprising at least two regions derived from the human T cell reactive feline protein or a peptide derived from the human T cell reactive feline protein, each of which has human T cell stimulating activity to the human T cell reactive feline protein and each of which does not bind immunoglobulin E specific for the human T cell reactive feline protein, or if binding to said immunoglobulin E occurs, such binding does not result in release of mediators from mast cells or basophils;

20 d) determining whether a specific Delayed Type Hypersensitivity reaction occurs; and

25 e) administering to the individual having a specific Immediate Type Hypersensitivity reaction and a specific Delayed Type Hypersensitivity reaction a therapeutically effective amount of a therapeutic composition comprising the modified form of the human T cell reactive feline protein or a portion thereof of step (c), or the human T cell reactive feline protein produced by recombinant DNA techniques of step (c), or the peptide of

30 step (c) and a pharmaceutically acceptable carrier or diluent.

80. An antibody specifically reactive with a peptide of claim 19.

81. The antibody of claim 80 which is a monoclonal antibody.

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82. A T cell clone specifically reactive with a peptide of claim 8.

83. A soluble T cell receptor specifically reactive with a peptide of claim 8.

84. An antibody specifically reactive with the T cell receptor of claim 83.

5 85. A multipeptide formulation suitable for pharmaceutical administration, comprising:
a first peptide of a cat protein allergen, human T cell reactive feline protein, having human T cell stimulating activity in an *in vitro* T cell proliferation assay and soluble at a physiologically acceptable predetermined pH;

10 a second peptide of a cat protein allergen, human T cell reactive feline protein, having human T cell stimulating activity in an *in vitro* T cell proliferation assay and soluble at the same physiologically acceptable pH as said first peptide; and an excipient.

15 86. The multipeptide formulation of claim 85, wherein the first peptide and the second peptide are lyophilized.

87. The multipeptide formulation of claim 85, wherein the excipient comprises sodium phosphate.

20 88. The multipeptide formulation of claim 85, wherein the excipient comprises mannitol.

89. The multipeptide formulation of claim 85, wherein the first peptide comprises an amino acid sequence represented in SEQ ID NO: 17 [Peptide X].

25 90. The multipeptide formulation of claim 85, wherein the second peptide comprises an amino acid sequence represented in SEQ ID NO: 18 [Peptide Y].

30 91. A therapeutic composition comprising at least one peptide of TRFP, at least one said peptide containing a sufficient percentage of the T cell epitopes present in TRFP to induce T cell nonresponsiveness in a substantial percentage of a population of individuals sensitive to TRFP.

35 92. A therapeutic composition comprising at least one peptide of claim 19, at least one said peptide comprising a sufficient percent of the T cell epitopes present in TRFP to induce T cell nonresponsiveness in a substantial percent of the population of

individuals sensitive to TRFP.

93. A therapeutic composition comprising at least one peptide of claim 42, at least one said peptide comprising a sufficient percent of the T cell epitopes present in TRFP to induce T cell nonresponsiveness in a substantial percent of the population of individuals sensitive to TRFP.

94. A method for treating sensitivity to a cat protein allergen in a subject sensitive to said allergen comprising orally administering a protein of claim 1, or any portion thereof.

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